PRELIMINARY INCUBATION OF RAW MILK SAMPLES AS AN AID IN DETECTING INSANITARY PRODUCTION PRACTICES

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INTRODUCTION

In the last few years rapid expansion of the practice of bulk milk handling has solved many difficulties at the production level. It is generally recognized that the quality of milk has been greatly improved by adoption of bulk cooling. Although the term "quality" is seldom defined, it is obvious that total bacterial count is the principal factor considered.

As a result of bulk tank handling of milk it appears that quality problems may be changing. When milk is cooled quickly and kept cold, bacterial growth is greatly retarded. Thus, efficient cooling may mask insanitary practices in production phases, by contributing to low bacterial counts (13). Standard bacteriological tests may fail to indicate such unsatisfactory conditions. Research workers in the industry have been concerned with the inadequacy of current methods and standards for measuring quality of fluid milk. As a consequence it has been proposed that raw milk samples be incubated at 55 F for 18 hr. before testing for bacteriological quality. It is contended that such preliminary incubation of raw milk samples before testing would encourage growth of contaminants and differentiate between milk that has been produced under sanitary conditions and that in which bacterial numbers have been suppressed by cooling. It is indicated that such procedure will give a more reliable measure of the care taken in the production and handling of milk and also it will contribute to better control of raw milk quality.

Investigations on the merits of the "preliminary incubation" procedure have been limited and confined to a few geographical areas. Further evaluation of the proposal is needed. Accordingly the work reported herein was undertaken to determine if, in North Central Kansas, the "preliminary incubation" procedure is a better indication of raw milk quality than the customary standard plate count on fresh milk samples. The study also was directed toward evaluation of seasonal influences and the effect of the preliminary incubation procedure on psychrophilic and coliform counts.

LITERATURE REVIEW

Effect of Bulk Cooling on Bacteriological Quality of Milk

Generally it has been acknowledged that a marked improvement in milk quality has resulted from the conversion of can cooling to bulk cooling at the production level. However, this largely depends upon the definition of quality. Normally it has been considered that the bacterial count is the chief criterion. Atherton (2) reported that more information is needed on the many conditions influencing the quality of milk cooled in bulk tanks.

It is known that prompt cooling of milk after production and maintaining at low temperature restricts bacterial growth. Milk so treated gives low counts by the standard plating method. Smillie et al. (20) suspected that efficient cooling of freshly produced milk might enable a milk to comply with regulatory bacterial standards by limiting microbial growth, even though the production methods might not meet desired standards of sanitation. According to Johns (14), at present, none of the bacteriological tests will differentiate between cleanly produced milk and that wherein careless practices are masked by superior cooling. He also stated that producers with bulk tanks might be careless in their sanitary production practices and still could comply with bacteriological standards. Davis and Killmeier (4) concluded that efficient cooling almost eliminated high bacterial counts. However, many producers continued to be degraded on the farms when farm inspectors found conditions not indicative of a high degree of cleanliness. As reported by Johns (14), Chalmers in Scotland also suspected that efficient cooling in farm bulk tanks might conceal insanitary practices at the production point of milk. From examination of 103 samples he found "probably about 24% of the samples gave initial counts (under 10,000/ml.) which did not reflect the probable hygienic conditions of production of the milk". From his experience Johns (12) reported that a reduction of the maximum standard for bacterial counts to 10,000/ml. would give no absolute assurance of thorough sanitation of the equipment and accessories of bulk tanks.

In the past it has been recognized that if thorough care is applied in the sanitary aspects of production, emphasis on cooling of milk could be reduced. Johns reported that this is due to the fact that the udder flora which comprises the bulk of bacteria in cleanly produced milk, grows best at body temperatures and multiplies very slowly at temperatures below 15 C (59 F). Trout et al. (24) found that fresh raw milk had a temporary inhibitory action on the growth of bacteria. This property was described by them as the "germicidal action of milk" and might be due, in part, to the normal lag phase characteristic of bacterial growth in a new environment. These workers also reported that the germicidal action of cow's milk was apparent rather than real, as the reduction in count of bacteria might be due to clumping of cells and to temporary dormancy rather than to actual dying of bacteria. They also showed that this germicidal power was entirely lost within a short time when milk was kept warm (98.6 F). The inhibitory property lasted from 3 to 6 hours at 70 F. It was less pronounced but more persistent at low temperature (40 F).

Fay (5) was of the opinion that the bacterial count is a valuable test, but it does not provide a complete measure of the quality of milk. Many insanitary practices which might occur during production of milk may not be detected with present bacterial standards. He felt that bacterial counts on producers' milk were greatly influenced by the ultimate cooling temperature and consequently might cover up unhygienic conditions to a profound extent. La Grange (16) could not establish a correlation between the standard plate counts on milk from individual producers and the score placed upon the milk production facilities largely because of excessive variation within several plate counts of the individual producers. In a pilot experiment, Orr et al. (18) found that on bulk milk farms where sanitary methods were not up to desired standards, low temperature cooling became a mask for unhygienic methods. They found that ordinary plate counts and coliform tests did not reflect the substandard sanitary practices that prevailed during production of cooled milk.

Limitations of Bacteriological Quality Tests on Bulk Milk

The changes in milk production techniques in recent years have influenced the bacterial flora of milk. Accordingly, useful quality tests of the past may be of less value under present conditions. Johns (10) believed that when the growth of organisms is effectively controlled by prompt and efficient cooling, tests presently designed for evaluating can milk quality might not be always effective in diagnosing faulty production practices in bulk milk handling. In view of this, he felt that reappraisal of bacteriological tests currently in use, was desired. He was also of the opinion that since pasteurized dairy products are being stored for longer duration in the period between processing and ultimate consumption, the importance of psychrophiles as postpasteurization contaminants is increased. Accordingly, tests on fresh samples are much less useful than those made after these organisms have been encouraged to develop in the product. Johns also concluded that specific groups of bacteria are important as indices of insanitary production and processing practices. He contended that with growth of coliform bacteria being hindered in bulk milk, their presence in pasteurized milk was of greater significance.

Davis and Killmeier (4) stated that low count (10,000 or less) raw milk could not possibly have a laboratory pasteurized plate count of excessive number. However, it might not have been produced under hygienic conditions. Storgrads (23) studied the relationship between producers' milk and market milk. He found that within wide limits the bacterial content of producers' milk had little effect on bacterial content of market milk. Schwarz and Giblis (19) studied results of bacteriological tests on farm milk supplies and market milk in a dairy during 1954-58. They found that the raw milk was generally unsatisfactory and that reinfection of pasteurized milk often occurred in the plant. Davis and Killmeier (4) reported that good cooling kept coliform counts low, but that low counts did not necessarily reflect good production practices.

Hempler (7) reported that the resazurin test, while useful in reflecting abnormal udder conditions, had limited value except where little quality improvement work had been attempted. He found that well cooled milk containing excessive numbers of dormant bacteria often escaped detection. Johns (10) reported the resazurin test as failing to detect a considerable percentage of high count milks from the bulk system. McCallum (17) studied bacteriological tests on Glasgow consumer milk. The chief tests used were plate counts and presumptive coliform tests. A comparison between the resazurin test and psychrophilic count was also made. The results indicated that the presumptive coliform test was the most reliable for detecting poor quality milk, the resazurin test being unreliable and the plate count being cumbersome to perform, as well as being subject to high error. Orr, et al. (18) reported from their studies that the resazurin test and the standard methylene blue test were no longer adequate as indicators of the quality of bulk milk.

Johns (10) reported that several factors were responsible for disturbing correlations between plate counts and reduction times of the methylene blue test in recent years. He stated that more efficient media and lower incubation temperatures have increased the level of plate counts. The proportion of thermoduric organisms also increased (21). These thermoduric organisms, as Johns (15) noted, are less active reducers. He also contended that psychrophiles which often comprise a high percentage of the flora fail to grow at 35-37 C. Finally, with prompt and efficient cooling, the bacteria are dormant in the beginning of the test and bacterial growth inhibiting substances are conserved. So, as a result of the prolonged lag phase, Johns (10) reported that the rate of dye reduction is much reduced and ultimately some high count milks escape detection. The methylene blue reduction test is not reliable when applied to high grade milks and it also suffers from the fact that in promptly cooled milk, the bacteriostatic activity may delay bacterial growth (9,11).

Preliminary Incubation of Raw Milk Samples as a Proposed Modification in Current Bacteriological Tests on Bulk Milk

Recognizing the growing need for a procedure which would reflect careless practices of handling bulk milk, Johns (9,10,11,12,13,14,15) suggested preliminary incubation (P.I.) of raw milk samples before testing as an aid in assessing the bacteriological quality of bulk milk. He concluded from his work that raw samples should be incubated at 55F for 18 hr. He argued that the udder flora which forms the bulk of organisms in cleanly produced milk grows best at body temperature and very slowly at temperatures below 15 C (59 F) (10), while saprophytic contaminants grow actively at 55 F. He proposed an 18 hr. holding period since it could be carried out overnight and conveniently fitted into laboratory schedules. Smillie, et al. (20) described the work of Chalmers who reported on plate count tests made on low count milk samples, a few hours after sampling and 21 hr. later. The milk samples were held at approximately 60 F. Chalmers showed that milk which gave initial counts of 10,000 or less per ml. at the first plating and no more than 21 times this initial count at plating after incubating samples could be predicted to be reasonably satisfactory. According to him, such milk can be judged as having been produced under good conditions.

Johns (11,9) showed that where the methylene blue test was used, it was quite necessary to have preliminary incubation of raw milk samples at 35.5 - 37.5 C for at least 6 hours. Hadland (6) found that after preliminary incubation of raw milk samples at 15 C for 15 to 20 hr. the methylene blue test gave an excellent indication of both total bacterial count and coliform count. Solberg and Bredholt (22) reviewed the work of Andersen who recommended that the milk samples should be incubated at 15 C to 18 C for 24 hr. before carrying out the reduction test as an improvement over present quality tests of bulk milk.

Effect of Preliminary Incubation on Bacterial Populations in Raw Milk Samples

Standard Plate Counts: Johns (14) showed that in almost every case where the ratio of the standard plate count after P.I. to initial count was high, the psychrophilic counts both initially and after P.I. were likewise high. However, this was not the case with coliform counts. He suggested that a standard plate count of 200,000/ml. following P.I. at 55 F for 18 hr. would be a better standard than would a lower initial count limit of 50,000/ml. Johns (15) also found that commercially produced raw milks varied greatly in their behavior following P.I. and in certain cases there was little or no change. In others there was a striking deterioration in quality while many were intermediate. He also observed that in sets of samples analyzed in July and November, two-thirds of the samples gave initial plate counts higher than the 50,000/ml. suggested limit for bulk milk. However, when milk was produced under controlled conditions, he concluded that milk drawn from clean cows, using clean milking machines, showed no deterioration after preliminary incubation. Failure to clean stalls, brush the cows and wash the udder before milking had surprisingly little influence on the bacteriological condition. Neglected milking machines played a prominent part in increasing bacterial contaminants. During February and July, the results were almost the same even though the level of initial count was quite low. Statistical analysis showed a highly significant correlation between utensil sanitation and growth during P.I.

Smillie, et al. (20) plated milk samples after incubating them for 6 hr. as well as for 30 hr. at mean atmospheric shade temperature and found wide relative increases in the counts of individual samples over initial counts of the same. Davis and Hillmeier (4) reported that when using P.I., 25% of their producers still ran a bacterial count of 200,000 or less. These farms which rated high in sanitation had initial counts of 10,000 or less. After P.I. these counts increased little, whereas in many other cases producers whose initial counts were 10,000 or less ran a count in excess of 200,000 and often in the millions. They suggested that producers whose counts remain below 200,000 after P.I. have high grade milk. Ayer, et al. (3) stored raw milk of three different grades at 40 F, 50 F, and 60 F. They found that samples of approximately the same initial bacterial count did not always show the same growth rate. Higher growth ratios were obtained in milk samples produced under dirty conditions in unsterilized utensils than in samples of milk produced under clean conditions in sterilized utensils. These workers considered that the bacteria which were introduced from unsterilized utensils grew faster at temperatures about 15.5 C (60 F) than did the bacteria in low count milk produced in sterilized utensils. Orr, et al. (18) also showed that P.I. counts gave larger increases with milk samples from insanitary farms. They concluded that ordinary plate counts did not assess the sanitary condition of bulk milk.

Coliform Counts: Fay (5) contended that when milk was being cooled promptly and efficiently in farm bulk tanks, the growth of coliform bacteria was minimized as compared to that in can cooled milk and that coliform counts on bulk milk might be expected to reveal more effectively the contamination from surroundings and equipment. Smillie, et al. (20,18) observed that room temperature incubation of milk samples for 6 hr. and 30 hr. had an influence on coliform results. They concluded that initial counts of coliforms did not assess the condition of bulk milk farms. Johns (14,15) found that in practically all cases, initial coliform counts were quite low, even though the original total counts and psychrophilic counts were high. He concluded that coliform organisms were not a suitable index of carelessness of milk production. Henningsen (8) found that when standard plate counts of < 50,000/ml. and coliform counts of < 100/ml. were adopted as standards of raw milk quality, there was little or no relationship between samples meeting the standards for total or coliform count. He suggested that the coliform count was an entirely different estimation of raw milk quality, than the total count.

Psychrophilic Counts: Johns (14) found that when the ratio of the standard plate count after P.I. to initial count was high, the psychrophilic counts both before and after P.I. were also high. Johns (15) also worked on commercially produced raw milks and found that many high counts of psychrophiles indicated poorly cleaned tanks. These high count samples generally showed considerable rise in count after P.I. Orr et al. (18) made counts for psychrophiles on original milk samples drawn from 13 farms and again counts were taken after incubating these samples at 5 C for 24 hr. The initial psychrophilic counts were low on the whole and only 5.5% of the samples after preliminary incubation failed a possible standard of 10,000/ml. However, samples from one farm, where production methods were unsatisfactory had an initial psychrophilic count < 10,000/ml., 20% of the samples after preliminary incubation exceeded this standard of 10,000/ml. A tendency for greater increase in P.I. counts of psychrophiles during summer months was observed as compared to increases in other seasons.

Thermoduric Counts: Johns (14) reported that in his study, thermoduric counts were all below 1,000/ml. He considered these organisms not sensitive enough to indicate carelessness in milk production. Thermoduric organisms would not be detected by P.I. since they fail to grow at 55 F. When Johns (15) later worked on commercially produced raw milks it was found that over one-half of the total samples showed a thermoduric count exceeding 1,000/ml. with a maximum of 100,000/ml., reflecting poor sanitation of milking machines. Even then his further studies on the same aspect revealed that thermoduric counts did not indicate the saprophytic contamination responsible for deterioration in quality of milk following P.I.

Reduction Tests: Johns (15), while working on commercially produced raw milks, found that after P.I., reduction of resazurin tests occurred in a number of samples in 3 hr. or less and there was much closer agreement between standard plate count and the resazurin test after P.I. Accordingly, he suggested that, following P.I., the resazurin triple reading test be substituted for the standard plate count method. When he worked on milk produced under controlled conditions, he reported that resazurin reduction times exceeded 4 hr. both before and after P.I. in all samples where clean milking machines were used. All samples drawn from neglected milking machines showed shorter reduction times. He concluded that there was a high correlation between sanitation and growth during P.I. Smillie, et al. (20) reported that dye reduction tests were of little value in assessing milk quality. Orr, et al. (18) incubated milk samples at mean atmospheric shade temperature not exceeding 18 C for 20 hr. to study the effects on resazurin tests and methylene blue tests. They concluded that this procedure was a more accuate measure of milk quality than such tests without P.I.

EXPERIMENTAL PROCEDURE

Collection of Samples

Milk samples were collected from 35 bulk tank grade-A producers in the Manhattan, Kansas, milk shed. Most of the samples were obtained by the county milk sanitarian and some were taken by the milk hauler who was trained and licensed to do such sampling. Although an attempt was made to obtain an equal number of samples from each producer during the different seasons of the year, this was not practical. Accordingly more samples were obtained during some seasons than in others. Samples were obtained aseptically after operating the bulk tank agitator for several minutes and were iced immediately after collection and brought to the laboratory. In almost all cases, samples were analyzed for inhibitory substances and for bacteriological quality promptly after arrival. Where it was necessary to hold the samples, they were kept at temperatures of 32 - 35 F for not more than 24 hours before analysis. General sanitary conditions of each producer's milk handling facilities were also judged and rated by the sanitarian as rank I, II and III with rank I being most satisfactory.

Analyses of Samples

Samples were analyzed for inhibitory substances in milk by the rapid disc assay screening method (1). Standard plate counts, coliform counts and psychrophilic counts were made initially as well as after incubating samples at 55 F (12.5 C) for 18 hr. The counts made after the incubation period were termed as preliminary incubation (P.I.) counts. Plating methods used were the standard procedures recommended by the American Public Health Association (1). For standard plate counts, plates were incubated at 35 C for 48 hr., whereas for coliform counts plates were incubated at 35 C for 20 hr. Violet red bile agar medium was used for coliform counts. For psychrophilic counts, plates were incubated at 7 C for 7 days.

In summarizing the data, standard plate counts and psychrophilic counts were averaged logarithmically as recommended in the Milk Ordinance and Code (25) to even out discrepancies. These counts are presented in results as geometric means. With coliform counts, aritimetic averages were used since the count range was much lower and some zero counts were obtained. Figures reported for counts are on a per ml. basis.

In studying the effect of season on preliminary incubation of raw milk samples the different seasons were considered as follows: Fall - September, October and November; Winter - December, January and February; Spring - March, April and May; Summer - June, July and August.

Statistical evaluations were made on some of the data by analysis of variance and linear regression.

RESULTS AND DISCUSSION

Tests for Inhibitory Substances

All milk samples but one gave negative results in tests for inhibitory substances. The one exception was classed as doubtful. Accordingly, it was concluded that the presence of inhibitory agents, as measured by the test employed, was not a factor in influencing bacterial development during the period of preliminary incubation of the milk samples.

Influence of Season on Standard Plate Counts Before and After Preliminary Incubation

Data have been summarized and grouped in Table 1, by season, to study the effect of preliminary incubation of milk samples at 55 F for 18 hr. on bacterial counts.

Table 1. Increase in standard plate count due to preliminary incubation of milk samples examined during different seasons.

		Geometric means of standard plate counts		
Season	No. of samples	Initial (A)	P.I. (B)	Growth ratio $\frac{B}{A}$
Fall	28	11,000	50,000	4.5
Winter	36	14,000	68,000	4.9
Spring	38	9,000	44,000	4.9
Summer	92	24,000	59,000	2.5
Summary	194	14,000[a]	55,000[a]	3.7

[a] Geo. avg. of seasonal means

The overall geometric average of initial standard plate counts was 14,000/ml. as shown in Table 1, which was much below the standard of 50,000/ml. adopted recently (14) in certain areas for raw bulk milk. Moreover, standard plate counts in fall, winter and spring averaged 14,000/ml. or below, with the summer counts averaging 24,000/ml. This presents some doubt as to whether the present standard of 200,000/ml. for raw bulk milk is too lenient or not consistent with changed conditions of handling milk. Various workers (13,12,10,5) have also expressed the same opinion.

The initial standard plate count averaged lowest in spring (9,000/ml.) and highest in summer (24,000/ml.). Fall and winter counts were in between. In the summer season a larger number of samples was analyzed than in other seasons. This might affect the results to some extent. However, it would be expected that the growth of bacterial contaminants in equipment would be greater in warmer weather.

Statistical analysis of the logarithms of individual counts (analysis of variance) showed that there was a significant effect ($P < .01$) of season on initial standard plate counts, with summer being significantly higher than fall or spring.

The overall geometric average P.I. count as shown in Table 1 was 55,000/ml. This would appear to be of importance since the initial count averaged relatively low. It was highest (68,000/ml.) in winter. The P.I. count was lowest (44,000/ml.) in spring. Fall and summer P.I. counts averaged in between the two extremes.

The overall average growth ratio due to P.I. was 3.7. Thus, it is evident that preliminary incubation has a definite effect in increasing the standard plate count. In summer the growth ratio due to P.I. was 2.5. It was the lowest of the four seasons. There was not much variation in growth ratios in fall (4.5), winter (4.9), and spring (4.9). Statistical analysis indicated that season had no effect on growth ratios due to P.I. in standard plate counts.

The foregoing results suggest that if the P.I. procedure were to be used as a milk quality measure, the effect of season on preliminary incubation of raw milk samples can probably be disregarded. However, during the summer the initial standard plate count would appear to be a good measure of quality.

During the other seasons the P.I. count is more effective in revealing organisms that do not show up in the standard plate count.

Influence of Season on Coliform Counts Before and After Preliminary Incubation

The data on coliform counts have been summarized and are presented on a seasonal basis in Table 2.

Table 2. Increase in coliform counts due to preliminary incubation of milk samples examined during different seasons.

		Arithmetic means of coliform counts		
Season	No. of samples	Initial (A)	P.I. (B)	Growth ratio $\frac{B}{A}$
Fall	27	313	2,100	6.9
Winter	32	55	56	1.0
Spring	37	220	1,400	6.4
Summer	92	1,700	4,000	2.3
Summary	188	570/a	1,900/a	3.3

/a Arith. avg. of seasonal means

The overall arithmetic average of initial coliform counts was 570/ml. The initial coliform count was highest (1,700/ml.) in summer and lowest (55/ml.) in winter, whereas spring and fall counts were within these limits. Relatively more seasonal variation occurred in coliform counts than in standard plate counts. This may be due to the use of arithmetic averages rather than geometric averages. The high coliform counts obtained in summer correlated with the high initial standard plate counts in summer. However, the relatively low coliform counts in winter raise some doubts as to whether coliform counts could be used as a measure of quality during all seasons.

The overall average growth ratio in coliform bacteria was 3.3. There was considerable variation between seasons. It was lowest (1.0) in winter and highest (6.9) in fall. Growth ratios in spring and summer were within this range. These results support the observations of Orr, et al. (18) and Smillie, et al. (20) that the P.I. procedure does have an effect on coliform counts but does not appear to be a suitable measure of the general bacteriological quality of milk, since the coliform growth ratio was lowest in winter, when the standard plate count after the P.I. was highest.

Influence of Season on Psychrophilic Counts Before and After Preliminary Incubation

A summary of the data on psychrophilic counts during the different seasons is given in Table 3.

Table 3. Increase in psychrophilic counts due to preliminary incubation of milk samples examined during different seasons.

		Geometric means of psychrophilic counts		
Season	No. of samples	Initial (A)	P.I. (B)	Growth ratio B/A
Fall	19	1,900	45,000	23.7
Winter	36	4,000	190,000	47.5
Spring	37	1,700	46,000	27.0
Summer	92	1,600	22,000	13.8
Summary	184	2,100[a]	54,000[a]	25.7

[a] Geo. avg. of seasonal means

The overall geometric average of initial counts of psychrophilic bacteria was 2,100/ml. It was highest (4,000/ml.) in winter and was lowest (1,600/ml.) in summer. Fall and spring counts were 1,900/ml. and 1,700/ml., respectively. Thus, it appears that in winter psychrophilic counts tend to be higher than in other seasons. This would not be unexpected since the prevailing lower temperatures would tend to encourage this type in the environment. However, statistical analysis showed that the differences observed due to season were not of statistical significance.

The overall geometric average P.I. count for psychrophilic bacteria was 54,000/ml. which was almost as high as the average P.I. standard plate count. The P.I. procedure had a much greater effect on psychrophiles than on standard plate counts or on coliform counts. This indicates that psychrophiles multiplied more rapidly during P.I. than did other types. The high standard plate counts obtained after P.I. in winter probably are due to greater increases of psychrophiles. However, although these obviously increased during P.I. (55 F for 18 hr.) it is very doubtful that they all grew at 95 F, standard plate count incubation temperature. The P.I. count averaged highest (190,000/ml.) in winter and lowest (22,000/ml.) in summer. It is significant that the P.I. psychrophilic count was much higher in winter than in other seasons and also much higher than the standard plate count. The results suggest that except in summer, the P.I. psychrophilic count is a better measure of bacteriological quality than the standard plate count. However, the length of time involved in such counts is a definite disadvantage and would limit the method for routine use.

The overall average growth ratio as shown in Table 3, was 25.7 for psychrophilic bacteria. The growth ratio in winter was highest (47.5) and in summer it was lowest (13.8). Compared to the overall average growth ratios in standard plate counts and coliform counts, the average growth ratio in psychrophilic bacteria was very high. The large difference in growth ratios between winter and summer may be due to sanitary conditions of farms and prevailing temperatures in winter and summer seasons. Statistical analysis of data showed that season had a significant ($P < .01$) effect on growth ratios of psychrophilic bacteria.

Relationship of Sanitary Rank of Producing Farms to Increase in Standard Plate Counts due to Preliminary Incubation

In Table 4, data have been grouped to study the effect of sanitary rank of farms on the increase in initial standard plate counts due to preliminary incubation of milk samples examined during different seasons.

The overall geometric means of initial counts for sanitation rank I, II and III were 12,000, 12,000 and 24,000 per ml. of milk, respectively. This suggests that rank III farms were definitely poorer in sanitary conditions. However, there was not much difference in overall geometric means of initial counts of rank I and rank II farms. This might be because farms were ranked by visual inspection and in such circumstances actual differences in sanitary conditions of milk handling equipment on rank I and rank II farms might be small. Nevertheless, geometric means of initial counts for the summer season in each of the sanitary ranks did not vary much. This might be due to prevailing high temperature in summer when bacteria multiply rapidly in equipment and hence tend to minimize differences in sanitation.

The overall geometric means of P.I. counts increased as sanitation rank increased. However, there was considerable variation between seasons and within sanitation ranks. Growth ratios did not show any consistent trend with sanitation ranks on inspection of data grouped in the table. However, they

Table 4. Relationship of sanitary rank of producing farms to increase in standard plate counts due to preliminary incubation of milk samples examined during different seasons.

	Sanitation rank I				Sanitation rank II				Sanitation rank III			
Season	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio B/A	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio B/A	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio B/A
Fall	10	8,600	29,000	3.3	8	10,000	140,000	14.0	8	16,000	45,000	2.8
Winter	10	14,000	63,000	4.5	17	11,000	43,000	3.9	8	25,000	130,000	5.2
Spring	12	5,800	18,000	3.1	16	8,400	39,000	4.7	8	32,000	110,000	3.4
Summer	38	25,000	63,000	2.5	39	21,000	59,000	2.3	14	26,000	73,000	2.4
Summary	70	12,000[a]	38,000[a]	3.2	80	12,000[a]	61,000[a]	5.0	38	24,000[a]	83,000[a]	3.45

[a] Geo. avg. of seasonal means

were lowest in summer for all the sanitation ranks. This might result from the high initial counts obtained in summer. As shown in previous tables the growth ratios usually are lower when initial counts are highest.

Relationship of Sanitary Rank of Producing Farms to Increase in Coliform Counts due to Preliminary Incubation

In Table 5, data have been grouped to show the effect of sanitary ranks of bulk milk farms on increases in initial coliform counts due to P.I.

The overall arithmetic means of initial coliform counts for sanitary ranks I, II and III were 300, 400 and 800 ml., respectively. The higher count for rank III farms is in agreement with the sanitary ranking and is additional evidence of inferior sanitary conditions. However, there was little difference in overall average counts for rank I and II. Within each of the sanitation ranks the initial coliform counts averaged highest in summer and lowest in winter. This indicates that prevailing temperatures in summer and winter might affect initial coliform counts.

The overall arithmetic means of P.I. counts for ranks I, II and III farms were 1,200, 3,300 and 11,000. This shows that the P.I. count increased as sanitation rank increased. The arithmetic means of P.I. counts did not show any consistent relation with seasons in the three sanitation ranks.

The overall average growth ratio increased as the sanitation rank increased. However, the average growth ratios in the different seasons varied considerably. They were lowest in summer in all sanitation ranks. This might be due to higher initial coliform counts in summer than in other seasons for each of the ranks. Although the overall P.I. averages and growth ratios appear to be related to sanitation ranks, it is questionable if they would be reliable measures of sanitation, due to seasonal variations involved.

Table 5. Relationship of sanitary rank of producing farms to increase in coliform counts due to preliminary incubation of milk samples examined during different seasons.

	Sanitation rank I				Sanitation rank II				Sanitation rank III			
Seasons	No. of samples	Arith. mean of initial counts (A)	Arith. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	No. of samples	Arith. mean of initial counts (A)	Arith. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	No. of samples	Arith. mean of initial counts (A)	Arith. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$
Fall	10	200	1,400	7.0	8	190	4,800	25.3	7	670	600	0.9
Winter	8	8	120	15.0	17	67	1,200	17.9	7	84	760	9.0
Spring	12	25	56	2.2	16	170	2,600	15.3	9	600	40,000	66.7
Summer	33	1,700	3,400	2.0	39	1,300	4,600	3.5	14	2,000	4,400	2.2
Summary	63	500/a	1,200/a	2.4	80	400/a	3,300/a	8.3	37	800/a	11,000/a	13.8

/a Arith. avg. of seasonal means

Relationship of Sanitary Rank of Producing Farms to Increase in Psychrophilic Counts due to Preliminary Incubation

In Table 6, data have been grouped to show relationships between sanitary ranks of farms and increase in initial psychrophilic counts due to preliminary incubation of milk samples during different seasons.

Overall geometric averages of initial counts for rank I, II and III were 1,200, 2,700 and 2,700 per ml., respectively. Geometric means of initial counts in each of the seasons increased as sanitary rank increased in most cases except in the summer season, where the average count remained generally similar. This suggests a relationship between initial psychrophilic counts and sanitation rank.

Initial psychrophilic counts tended to be slightly higher in winter. However, analysis of data in Table 6 indicated that the observed differences in initial count due to season and sanitation rank were not statistically significant.

Overall geometric means of P.I. counts were 26,000, 35,000 and 110,000 for sanitary ranks I, II and III, respectively. These results show that the P.I. count increased as the sanitary rank increased. P.I. counts in the winter season were highest.

Overall growth ratios for rank I, II and III were 21.7, 12.9 and 40.7. Thus, the overall growth ratio did not show a consistent relationship with sanitation ranks although it was highest for rank III. Growth ratios in each sanitation rank were highest in the winter season and also highest in sanitation rank III. The effects of season and sanitation rank on growth ratios were found to be statistically significant. ($P < .05$)

Table 6. Relationship of sanitary rank of producing farms to increase in initial psychrophilic counts due to preliminary incubation of raw milk samples in different seasons.

	Sanitation rank I				Sanitation rank II				Sanitation rank III			
Seasons	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio B/A	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio B/A	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio B/A
Fall	5	700	14,000	20.0	7	5,000	10,000	2.0	5	1,000	21,000	21.0
Winter	10	3,000	160,000	53.3	18	3,600	140,000	38.9	8	6,000	500,000	83.3
Spring	12	700	12,000	16.1	16	1,600	36,000	22.5	9	6,600	410,000	62.1
Summer	37	1,300	16,000	12.3	39	1,900	30,000	15.8	14	1,300	29,000	22.3
Summary	64	1,200/a	26,000/a	21.7	80	2,700/a	35,000/a	12.9	36	2,700/a	110,000/a	40.7

/a Geo. avg. of seasonal means

It appears that P.I. counts of psychrophiles are an index of sanitation. However, as pointed out earlier, the time involved limits the value of such a procedure.

Relationship of Range of Initial Standard Plate Counts and Sanitary Rating of Farms to Preliminary Incubation Counts

Summarized data are presented in Table 7, indicating the trend of growth ratios with level of initial counts and sanitation ranks.

In ranks I, II and III 48%, 41% and 40%, respectively, of the samples had initial counts less than 10,000. Geometric mean counts of these samples were 4,900, 4,600 and 5,400 in sanitation rank I, II and III, respectively. This indicates that milk with relatively low initial standard plate counts (<10,000/ml.) is not necessarily limited to that produced on farms with sanitation rank I. Moreover, 77%, 84% and 62.5% of the samples in sanitation rank I, II and III, respectively, had counts of 50,000 or less. This suggests, as pointed out previously, that the present standard of 200,000/ml. for raw bulk milk is exceptionally lenient.

In all count ranges, the P.I. count increased as initial count increased, and this relationship occurred in all sanitation ranks. However, the actual growth ratios did not show a uniform relationship. It is significant that in all ranges of initial counts up to 50,000/ml., the growth ratios increased as sanitation rank increased. Statistical analysis of the logs of individual counts by the linear regression method also showed that as sanitation became poorer, the growth ratios became greater.

In view of the relationships of P.I. count and growth ratio with sanitary conditions of farms, it appears that the P.I. count would be a better measure of sanitary conditions of bulk milk producing farms, than the normal standard

Table 7. Relationship of range of initial standard plate count and sanitary rating of farms to preliminary incubation counts.

Range of initial standard plate count	Sanitation rank I				Sanitation rank II				Sanitation rank III			
	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$
<10,000	33	4,900	15,000	3.1	34	4,600	20,000	4.3	16	5,400	31,000	5.7
10,000 to 30,000	17	16,000	52,000	3.3	27	18,000	79,000	4.4	9	18,000	91,000	5.1
30,000 to 50,000	5	39,000	79,000	2.0	6	36,000	77,000	2.1	3	37,000	310,000	8.4
>50,000	14	150,000	290,000	1.9	12	120,000	430,000	3.6	12	160,000	460,000	2.9

plate count procedure. However, with high count milk ($>$50,000) there is less relationship between growth ratio and sanitation rank. Hence, with such milk the regular standard plate count is as good a measure of sanitation as the P.I. count. In other words, with milk of low initial count, the P.I. procedure helps to detect undesirable sanitary conditions.

Relationship of Range of Initial Coliform Counts and Sanitary Rating of Farms to Preliminary Incubation Counts

Data are presented in Table 8, to show the relationship of range of initial coliform counts and sanitary rating of farms to increases in counts due to P.I.

In rank I, II and III, 34%, 20% and 12% of the samples, respectively, had initial coliform counts $<$ 10. This indicates that the better the sanitary conditions of farms the larger the percentage of samples with low initial coliform counts.

In each of the ranks the mean P.I. counts increased as the mean initial counts increased. However, growth ratios did not show consistent relationships with either initial count ranges or sanitation ranks. On the other hand as observed in earlier data, growth ratios tended to be lowest with the highest initial counts.

Thus, it appears that neither initial nor P.I. coliform counts are a satisfactory measure of sanitary conditions of farms irrespective of initial count ranges.

Table 8. Relationship of range of initial coliform counts and sanitary rating of farms to preliminary incubation counts.

Range of initial coliform counts	Sanitation rank I No. of samples	Arith. mean of initial counts (A)	Arith. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	Sanitation rank II No. of samples	Arith. mean of initial counts (A)	Arith. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	Sanitation rank III No. of samples	Arith. mean of initial counts (A)	Arith. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$
<10	24	6	47	7.8	18	3	100	33.3	4	4	42	10.5
10 to 100	20	41	1,100	26.8	22	35	760	21.7	16	31	700	22.5
100 to 1,000	12	450	2,000	4.4	23	350	4,200	12	8	370	4,500	12.1
>1,000	13	4,900	7,600	1.5	12	3,900	14,000	3.5	6	5,700	11,000	1.9

Relationship of Range of Initial Psychrophilic Counts and Sanitary Rating of Farms to Preliminary Incubation Counts

Summarized data have been grouped in Table 9, to present the relationship of range of initial psychrophilic counts and sanitary ranks with growth ratios due to P.I.

In rank I, II and III, 48%, 44% and 20% of the samples, respectively, had initial psychrophilic counts less than 1,000/ml. This indicates that the better the sanitary conditions of farms, the larger the percentage of samples with low initial psychrophilic counts. It also appears that milk with low initial psychrophilic counts is not limited to farms with best production methods.

In most cases P.I. counts increased as sanitary rank increased. However, growth ratios did not show a consistent relationship with sanitation ranks. Although growth ratios increased as sanitation rank increased in low count milks ($<$ 1,000/ml.) variations were found in other ranges. Nevertheless, growth ratios generally were highest in rank III. Accordingly, it appears that when initial psychrophilic counts do not exceed 50,000, either P.I. psychrophilic counts or growth ratios would be an index of sanitation.

Table 9. Relationship of range of initial psychrophilic counts and sanitary rating to preliminary incubation counts.

Range of initial psychro-philic counts	Sanitation rank I				Sanitation rank II				Sanitation rank III			
	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$	No. of samples	Geo. mean of initial counts (A)	Geo. mean of P.I. counts (B)	Growth ratio $\frac{B}{A}$
< 1,000	31	870	6,200	7.1	35	370	9,000	24.3	10	190	9,600	50.5
1,000 to 5,000	19	1,800	30,000	16.7	18	2,700	26,000	9.6	10	1,900	63,000	33.2
5,000 to 10,000	1	9,200	71,000	7.7	12	7,600	280,000	36.8	6	7,000	230,000	32.9
10,000 to 50,000	12	19,000	130,000	6.3	11	23,000	360,000	15.6	7	21,000	670,000	31.9
> 50,000	2	75,000	10,000,000	133.3	4	100,000	4,400,000	44.00	3	590,000	15,000,000	25.4

SUMMARY AND CONCLUSIONS

Studies were made to evaluate the "preliminary incubation" procedure (P.I.) of raw milk samples as a measure of production sanitation. The investigation was conducted on bulk tank milk from grade A dairy farms in the Manhattan, Kansas, milk shed. A total of 194 milk samples were obtained from 35 farms during four different seasons. Both initial and P.I. counts were made for total bacteria (SPC), coliforms and psychrophiles. Tests were also made for the presence of inhibitory substances. Farms were ranked I, II and III on the basis of general visible sanitation with rank I showing most evidence of desirable sanitary conditions.

With the exception of one doubtful sample, all tests for inhibitory substances were negative. Hence, it is probable that P.I. counts were not affected by inhibitory agents.

The overall geometric average of initial standard plate counts was 14,000/ml. This suggests that the current standard of 200,000/ml. is exceptionally lenient when applied to bulk tank milk. Initial counts were significantly higher during the summer season. However, P.I. counts increased proportionately more during the other seasons. It would appear that during the summer the initial standard plate count is as good a measure of bacterial content as the P.I. count. In other seasons the P.I. count seemed to be more effective in this respect. In relating bacterial counts to sanitation standards, statistical analysis showed that as sanitary conditions became poorer the growth ratio due to P.I. became greater. Hence, the P.I. count was generally a better indication of farm sanitation than the initial count. This was particularly true in lower count ranges. In the higher count ranges the P.I. count was no better measure of sanitation than the initial count.

The initial coliform counts averaged highest, (1,700/ml.), in summer and lowest, 55/ml., in winter. This indicated that prevailing temperatures in summer and winter might affect initial coliform counts. Relatively more variation occurred in coliform counts as compared to standard plate counts. Also comparatively low initial and P.I. counts in winter raise some doubt as to whether coliform counts could be used as a measure of bacteriological quality during all seasons. P.I. counts increased as sanitation rank increased. However, P.I. counts did not show any consistent relation with seasons and sanitation ranks. Thus, it appeared that neither initial nor P.I. coliform counts were a satisfactory measure of sanitary conditions of farms irrespective of initial count range.

It appeared that milks with low initial psychrophilic counts are not limited to farms with best production methods. Statistical analysis also showed that differences in initial psychrophilic counts due to sanitation ranks were not significant. In winter psychrophilic counts tended to be higher. However, statistical analysis showed that seasons had no significant effect on initial counts of psychrophilic bacteria. The P.I. procedure had a much greater effect on psychrophilic bacteria than on standard plate counts or coliform counts. The P.I. psychrophilic count was lowest in summer and also was highest in winter. The average growth ratio for psychrophilic bacteria was very high as compared to the growth ratio for standard plate counts and coliform bacteria. Effect of sanitation rank on growth ratio was also found to be statistically significant. However, growth ratios varied much between winter and summer. Statistical analysis showed that season had a definite effect on growth ratios of psychrophilic bacteria. In general it would appear that in counts up to 50,000/ml., P.I. psychrophilic counts would be a possible indication of farm sanitation except in summer. However, the length of time involved would limit the practicability of the method.

The general conclusion of this study is that except during summer, P.I. standard plate counts would be a better measure of farm sanitation than customary standard plate counts, coliform or initial psychrophilic counts. Although the P.I. psychrophilic count appears to be the best measure of sanitation and bacteriological quality, it requires too much time to be of practical value.

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PRELIMINARY INCUBATION OF RAW MILK SAMPLES AS AN AID IN DETECTING INSANITARY PRODUCTION PRACTICES

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Recent reports suggested that efficient cooling may mask insanitary practices in the production phases of bulk tank milk by greatly retarding bacterial development. Consequently, low bacterial counts on such milk are not necessarily indicative of good sanitary conditions. It also has been proposed that preliminary incubation of raw milk samples at 55 F for 18 hr. before testing for bacteriological quality would aid in detecting milk produced under questionable sanitary conditions. It was postulated that contaminating organisms from poorly cleaned equipment multiply more rapidly at 55 F than do types from other sources such as the udder. Hence high P.I. (preliminary incubation) counts would reflect insanitary practices. Since previous investigations into the P.I. procedure have been limited, the present study was undertaken to further evaluate the method as a measure of production sanitation.

A total of 194 milk samples were obtained from 35 bulk tank grade A milk producers in the Manhattan, Kansas, milk shed during different seasons. These were analysed for numbers of total bacteria (SPC), coliforms and psychrophiles both before and after P.I. The samples were also tested for the presence of inhibitory substances. The general sanitary conditions of each producer's milk handling facilities were also rated as rank I, II and III with rank I being the best.

Except for one doubtful sample, all tests for inhibitory substances were negative. The overall geometric mean of initial standard plate counts was 14,000/ml. This presented some doubt as to whether the present bacterial standard of 200,000/ml. for raw bulk milk is consistent with changed conditions of milk handling. Statistical analysis showed that there was a significant effect of season on initial standard plate counts with summer being highest. However, P.I. counts increased proportionately more during the other seasons. It would appear that during the summer the initial standard plate count is as good a measure of bacterial quality as the P.I. count. In other seasons the P.I. count seemed to be more effective in this respect. In relating bacterial counts to sanitation rank statistical analysis showed that as sanitary conditions became poorer the growth ratio (P.I. count/initial count) due to P.I. became greater. Hence, the P.I. count was generally a better indication of farm sanitation than the initial count. This was particularly true in lower count ranges. In the higher count ranges the P.I. count was no better measure of sanitation than the initial count.

As compared to standard plate counts relatively more variation occurred in coliform counts. It is doubtful whether coliform counts could be used as a measure of bacteriological quality during all seasons considering the comparatively low initial and P.I. counts of coliforms in winter. Although P.I. counts increased as sanitation rank increased, there was no consistent relation with seasons and sanitation ranks. It appeared that neither initial nor P.I. coliform counts were a satisfactory measure of sanitary conditions of farms irrespective of initial count range.

Milk with low initial psychrophilic counts was not limited to farms with the best production methods. Statistical analysis also showed that differences in initial psychrophilic counts due to sanitation ranks were not significant. Although psychrophilic counts were higher in winter, statistical analysis showed that season had no significant effect on initial counts of psychrophilic bacteria. Psychrophilic counts were influenced much more by the P.I. procedure than were standard plate counts or coliform counts. The P.I. psychrophilic counts were lowest in summer and highest in winter. The mean growth ratio for psychrophilic bacteria was very high compared to the growth ratio for standard plate counts and coliform bacteria. It was also found that the effect of sanitation rank on growth ratio was statistically significant. However, growth ratio varied much between winter and summer. Statistical analysis also showed that season had a definite effect on growth ratios of psychrophilic bacteria. In general it would appear that in milk with initial counts up to 50,000/ml., P.I. psychrophilic counts would be an index of farm sanitation except in summer. However, an important limitation in the applicability of the method is the length of time involved.

The general conclusion of this investigation is that, except during summer, P.I. standard plate counts would be a better measure of farm sanitation than customary standard plate counts, coliform or initial psychrophilic counts. Although the P.I. psychrophilic counts appear to be the best measure of sanitation and bacteriological quality, it requires too much time to be of practical value.